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A Unique Model Platform for C4 Plant Systems and Synthetic Biology

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THE UNIVERSITY OF QUEENSLAND

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Final Report for AOARD Grant 144028 A Unique Model Platform for C4 Plant Systems and Synthetic Biology

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Period of Performance: 3 June 2014 – 2 June 2015

Abstract: We are developing *Setaria viridis* as a C4 model to accelerate metabolic engineering of key C4 crop species. A genome scale metabolic reconstruction was produced using our C4GEM framework. The reconstruction accounted for 1230 genes and 1588 reactions. The reconstruction was used to guide the comparison of transcriptome, proteome and central metabolome in mature and immature tissue. Preliminary data were obtained suggesting successful agrobacterium mediated transformation.

Introduction: C4 plants such as sugarcane, maize and sorghum are more efficient at carbon fixation than C3 plants. We have previously demonstrated that C4 plants can be engineered for sustainable chemicals production. However, C4 crop plants have large polyploidy genomes, poor transformation efficiency and long cycle times, leading to very long development times. In this project, we sought to develop a unique, versatile and powerful systems and synthetic biology platform for metabolic engineering of C4 plants. The specific aims were to develop

1. a genome scale metabolic reconstruction for Setaria;
2. a spatio-temporal molecular inventory during growth; and
3. a simple dipping protocol for Setaria transformation

Experiment:

Aim 1. A genome scale metabolic reconstruction was developed from the Setaria genome using our previously published C4GEM reconstruction framework.

Aim 2. Plants were grown from seeds in a growth chamber and a growth curve constructed. Immediately prior to the plants entering flowering state, biomass was harvested and split into mature and immature tissue for subsequent analysis. mRNAs were extracted from triplicate samples and submitted to the Ramciotti Centre at UNSW for RNA-seq analysis. Proteins were extracted from triplicate samples and analysed internally using a SWATH protocol on an AB Sciex 5600. The level of central carbon metabolites (53) was determined for pentaplicate samples using an in-house protocol of ion-pairing chromatography followed by SRM analysis on an AB Sciex 4500.

Aim 3. Agrobacterium-mediated transformation of Setaria viridis. Agrobacterium tumefaciens strain GV3101 was transformed by electroporation with pBI 121. Agrobacterium containing pBI 121 suspended in 50 mM potassium phosphate, pH 7.5 supplemented with 0.2-1 mM acetosyringone (MW 196.2, stock solution 0.1M in DMSO) was injected into the space immediately above developing inflorescence and the resulting seeds were characterized for GUS expression.

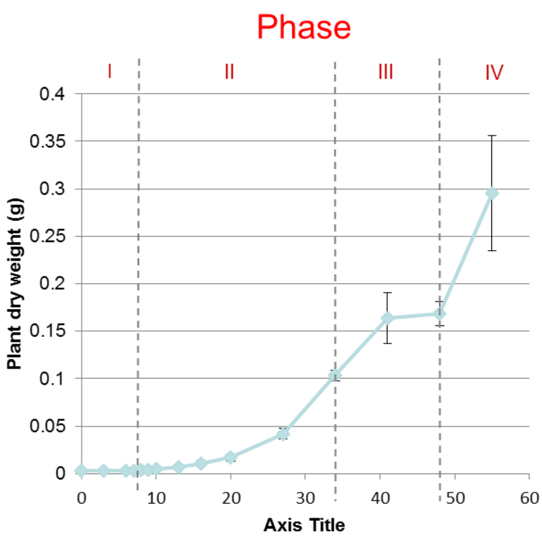
Results and Discussion:

Aim 1. A genome scale metabolic reconstruction for Setaria

Elements	***C4 plant models***			
	Sorghum bicolor	***Zea mays***	***Saccharum officinarum***	***Setaria viridis***
ORF-reaction-association entries	13114	38892	13593	8168
ORFs (unique genes)	3557	11623	3881	1230
Metabolites	1755			1755
Unique reactions	1588			1588
Extra cellular transporters	18			19
Transporters (plasmodesmata)	7			7
Transporters (Inter-organelle)	83			83
Biomass drains	47			48
GAP filling from *AraGEM*	131	135	156	186

A genome scale metabolic reconstruction was produced from the *Setaria viridis* genome. A total of 1230 genes were mapped to 1588 unique reactions. 186 reactions essential for growth had to be gap filled from Arabidopsis suggesting that the gene model for *Setaria* remains incomplete.

Aim 2. Spatio-temporal molecular inventory during growth



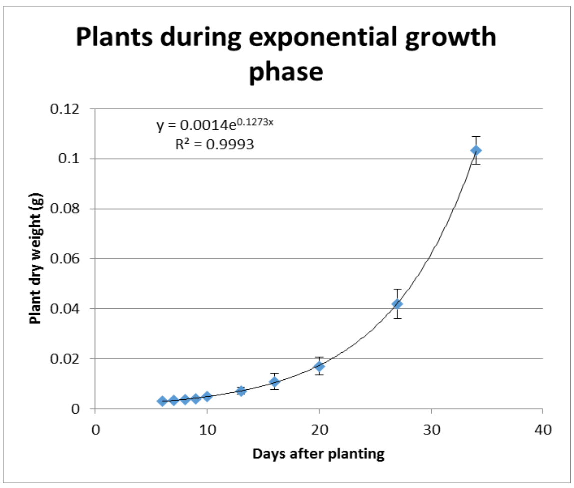


Figure 2. *Left panel:* Phase I (Day 0-6): Increase in FW but no increase in DW - seed imbibing water, seed resources used to generate initial seedling. Phase II (Day 6-34): Photosynthesis begins, dry weigh increases exponentially. Phase III (Day 34-48): Plant transitioning to flowering state. Phase IV (Day 48-55): Photosynthate devoted to developing flowers. *Right panel*: Exponential growth phase with a growth rate of 0.127 h^{-1}.

Biomass was harvested a 30 hours, split in mature and immature internodes, and subjected to comprehensive omics analysis. Of 35,425 genes expressed, 15,788 were observed to be differentially expressed between mature and immature internodes. The large set of expressed genes was used to expand the annotated gene list in the genome. Similarly, 190 out of 570 proteins determined by SWATH were differentially expressed, 20 out of 53 central carbon metabolites were differentially expressed. The data showed the expected down regulation in lipid biosynthesis in mature tissue, but changes to other functions was more subtle. Using the genome scale reconstruction for analysis, evidence was found for a change in the C4 mechanism from NAD-ME linked in immature to NADH-ME linked in mature tissue. This observation is in line with increasing evidence of a fluid transition between the different C4 types.

Aim 3. Agrobacterium-mediated transformation of Setaria viridis

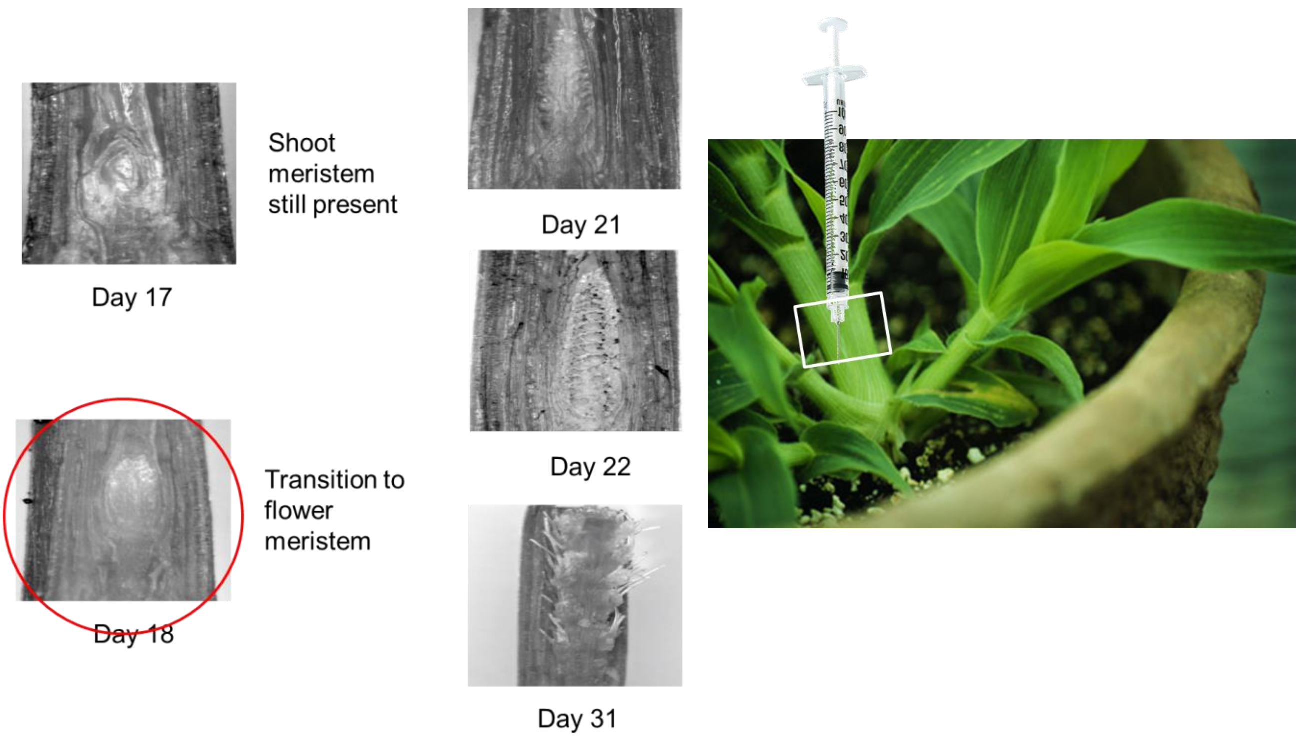


Figure 3. *Left panel*: Setaria meristems where sectioned longitudinally at daily intervals after

day 14 to determine onset of floral embryogenesis. Floral development commenced at about day 18 under our growth cabinet conditions (12 hour light – 28C, 12 hour dark – 24C). *Right panel:* Agrobacterium injected just above developing flower meristem on day 18. The inoculated shoot was tagged because new shoots emerge over time and it can be difficult to identify the original inoculated shoot.

An agrobacterium-mediated transformation protocol was developed for *Setaria viridis*. Onset of floral embryogenesis was determined to be around Day 18 and agrobacterium was injected just above the developing floral meristem (Figure 3). Preliminary data for Setaria transformation were very promising (Figure 4). However, we still need to test if introduced traits are stable across generations. Setaria transformation work will continue at UNT using fluorescent proteins or a seed colour gene to identify transformants.

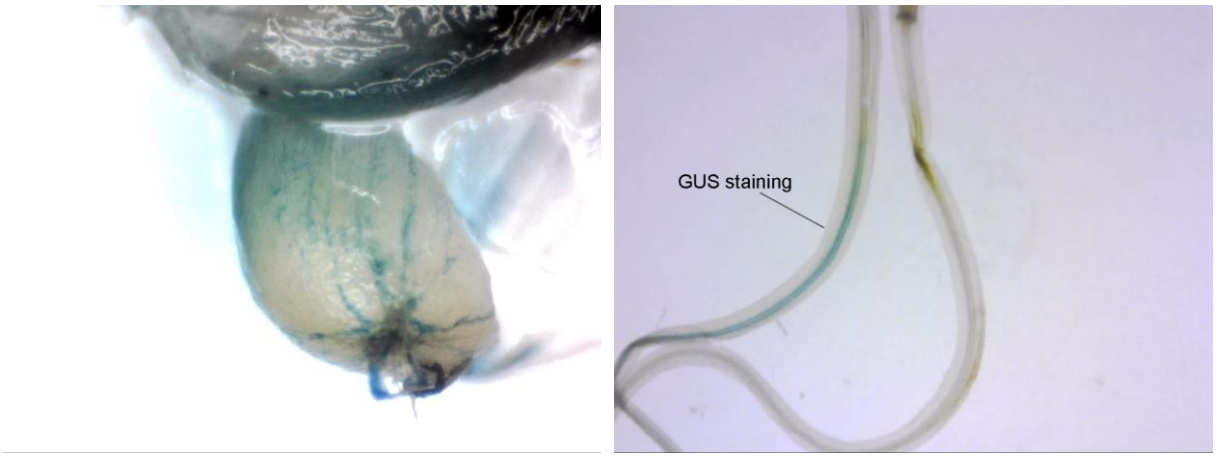


Figure 4. Seeds germinated and stained with GUS. *Left panel:* GUS staining was observed in the seed coat. *Right panel:* GUS staining was also present in the elongation zone of shoots in some seedlings.

List of Publications and Significant Collaborations that resulted from your AOARD supported project: In standard format showing authors, title, journal, issue, pages, and date, for each category list the following:

a) Papers published in peer-reviewed journals: N/A

b) Papers published in non-peer-reviewed journals or in conference proceedings: N/A

c) Conference presentations

- Nielsen LK (2014) Systems Biology [Plenary talk] International Conference in Bioinformatics, Sydney, Australia, July 31 - August 2, 2014.
- Nielsen LK (2015) Genome scale metabolic and regulatory network modelling in higher eukaryotes [invited talk]. Peking University, 16 March 2015.

d) Manuscripts submitted but not yet published: N/A (manuscript in preparation)

e) Provide a list any interactions with industry or with Air Force Research Laboratory scientists or significant collaborations that resulted from this work.

- Nielsen LK (2015) Multi-tissue genome scale modelling: toward understanding plant metabolism at an organismal level [invited talk]. Bayer, Ghent, 28 May 2015.
- Dr Richard McQualter has moved to University of Northern Texas and will continue working with Setaria under the guidance of Dr Stevens Brumbley.

DD882: As a separate document, please complete and sign the inventions disclosure form.